1: J Pathol. 1998 Nov;186(3):275-80. Related Articles, Lin

Expression patterns of the human papillomavirus type 16 transcription factor E2 in low- and high-grade cervical intraepithelial neoplasia.

Maitland NJ, Conway S, Wilkinson NS, Ramsdale J, Morris JR, Sander: CM, Burns JE, Stern PL, Wells M.

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Specific antibodies against the C-terminus of E2, produced by affinity purification of polyclonal antisera, have been used to identify the cellular populations which express the HPV 16 E2 transcription factor, in a series of formalin-fixed, paraffin-embedded cervical tissues. Cases were selected for both the presence of HPV 16 DNA (confirmed by multiple gene-specific PCl detections) and the presence of multiple grades of cervical intraepithelial neoplasia (CIN). The data indicate that E2 expression is highest in CIN I and in koilocytic lesions. Lower expression was observed in CIN II and little in CIN III lesions. In contrast, there was some restoration of E2 expression in invasive carcinomas, although the intracellular distribution was much more diffuse. The location of E2 expression to the superficial layers of the cervical epithelium, as well as the occurrence of some basal expression in CIN I, suggests that antibodies against HPV 16 E2 could be a useful adjunct to standard histological techniques for the detection of 'at-risk' patients as part c a cervical screening programme.

PMID: 10211116 [PubMed - indexed for MEDLINE]

EXHIBIT A

1: Protein Expr Purif. 2003 Jul;30(1):1-10. Related Articles, Lin

ELSEVIER FULL-TEXT ARTICLE

Overexpression, purification, and structural analysis of the hydrophobic E5 protein from human papillomavirus type 16.

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The E5 proteins of human papillomavirus (HPV) are highly hydrophobic transmembrane proteins that display weak transforming activity. The HPV E proteins are localized largely to intracellular membranes, such as the Golgi apparatus and endoplasmic reticulum, but also appear in the plasma membrane. Infection with HPV16 is the cause of over 90% of human cervica cancers. HPV E5 is known to interact with growth factor receptors and gap junction proteins and is believed to play a role during the initiation of neoplasia. The structure of HPV E5 and the mechanism of its interactions wi growth factor receptors remain largely unknown. In the present studies, the E protein of HPV16 was cloned into the pBAD/TOPO vector fused to an N-terminal thioredoxin leader and a C-terminal His-tag, and expressed in Escherichia coli. The identity of the protein was confirmed by immunoblottir using antibodies against a V5-epitope tag engineered into the protein. Due to formation of high molecular mass superaggregates of the protein, two chromatography steps were employed for its purification: (1) gel filtration chromatography to separate the superaggregated protein from other soluble proteins and (2) Ni-chelate affinity chromatography in the presence of detergent. The superaggregates of the E5-fusion protein were broken down tc monomers and various oligomers by sonication in the presence of 0.2% SDS The purified E5-fusion protein was then reconstituted into lipid vesicles and initial structural analysis of the protein was performed using circular dichroism spectroscopy.

PMID: 12821315 [PubMed - in process]